



BRIEF COMMUNICATION

ExGen 500 is an efficient vector for gene delivery to lung epithelial cells *in vitro* and *in vivo*

S Ferrari¹, E Moro¹, A Pettenazzo¹, JP Behr², F Zacchello¹ and M Scarpa¹

¹Department of Pediatrics and CRIBI Biotechnology Center, University of Padova, Italy; and ²Laboratoire de Chimie Génétique, Université L. Pasteur, Faculté de Pharmacie, Illkirch, France

Nonviral vectors might represent a safe alternative to adenovirus for gene therapy of lung disorders, in particular cystic fibrosis (CF). Cationic lipids have been shown to correct the CF defect both *in vitro* and *in vivo*, but more efficient vectors are needed to improve the low gene transfer efficiency. Here, we show that the cationic polymer ExGen 500, a linear polyethylenimine derivative, is more efficient than cationic lipids in transferring reporter genes to lung epithelial cells *in vitro*. *In vivo* ExGen 500 was able to mediate gene transfer into both newborn and adult rabbit lungs with comparable efficiencies. The best levels of

transfection were obtained using neutral complexes. Under such conditions, luciferase activities corresponding to about 10^3 RLU/10 s/mg of protein were reproducibly obtained 2 days after transfection throughout the four lung lobes of newborn and adult rabbits. A *nlacZ* reporter gene showed transfected cells around the lumen of large and small bronchi. No signs of acute toxicity (inflammation, cellular infiltration etc) were detected by direct histopathological analysis. Within 1 week after instillation, transgene expression decreased by two orders of magnitude.

Keywords: gene therapy; cystic fibrosis; synthetic vectors; lung

Since the cloning of the cystic fibrosis (CF) gene in 1989,^{1,2} encouraging results towards the gene therapy of this disease have been obtained. The observation that cystic fibrosis transmembrane conductance regulator (CFTR) gene transfer corrects the CF bioelectric defect in many model systems stimulated the development of various molecular vectors, effective in transducing the most affected tissue, the lung. Adenoviruses were the first vectors to be used for the gene therapy of CF, because of their ability to transfect nonreplicating cells and their tropism for the respiratory airways. Phase I CF clinical trials showed that adenoviral vectors might be successful in transferring the normal CFTR cDNA, although they may cause inflammation and immunological response.³ New viral vectors are now available; however, their ability to escape the immune response has still to be fully demonstrated.⁴

Recently, synthetic vectors have been suggested as an alternative to replication defective viruses. Their major advantage is the lack of inflammatory reaction or immunological response. Phase I clinical trials involving synthetic molecules (DC-Chol, DMRIE) were recently performed in the USA and Europe.^{5,6} Preliminary results confirmed the safety of these compounds, although their low gene transfer efficiency stressed the need for the development of more efficient molecules.

Among nonviral vectors two main classes of molecules can broadly be distinguished: cationic lipids such as Lipofectin, DOTAP or DOGS, and polymeric DNA-binding cations such as poly-L-lysine, protamine and polyethylenimine (PEI) (Figure 1).

The commercially available Lipofectin is a 1:1 mixture of DOTMA and DOPE, a neutral lipid. Lipofectin has been shown to deliver reporter genes to the rodent airways via direct intratracheal injection^{7,8} or intravenous administration of lipid-DNA complexes.⁹ Moreover, Lipofectin-DNA conjugates were able to mediate CFTR cDNA transfer to epithelial cells *in vivo* with reversal of the ion transport defect in transgenic CF mice.¹⁰ DOTAP is a monocationic lipid that was able to transfer efficiently the *lacZ* reporter gene or CFTR cDNA into mice, without any inflammatory response.¹¹ The lipopolyamine DOGS (Transfectam) is characterized by a polycationic DNA-compacting headgroup, spermine.¹² We showed previously that DOGS/DOPE was able to transfer marker genes into the mouse upper airways after intratracheal instillation.¹³ However, the low efficiency of transfection, despite the large amount of DNA used, together with the difficulty of reproducing results in independent experiments, led us to evaluate whether a new compound, ExGen 500, might be an alternative for *in vitro* and *in vivo* gene transfer to lung cells. ExGen 500 is a linear 22 kDa form of polyethylenimine, an organic polymer with a high cationic charge density potential. Similar branched molecules (PEI 800 kDa and PEI 25 kDa) were recently used to transfer marker genes to the newborn and adult mouse brain.^{14,15} Here, we first compared ExGen 500 to Lipofectin, DOTAP and DOGS on CF epithelial cell lines

Correspondence: M Scarpa, Department of Pediatrics and CRIBI, University of Padova, Via Colombo 3, 35121 Padova, Italy
Received 24 March 1997; accepted 6 June 1997

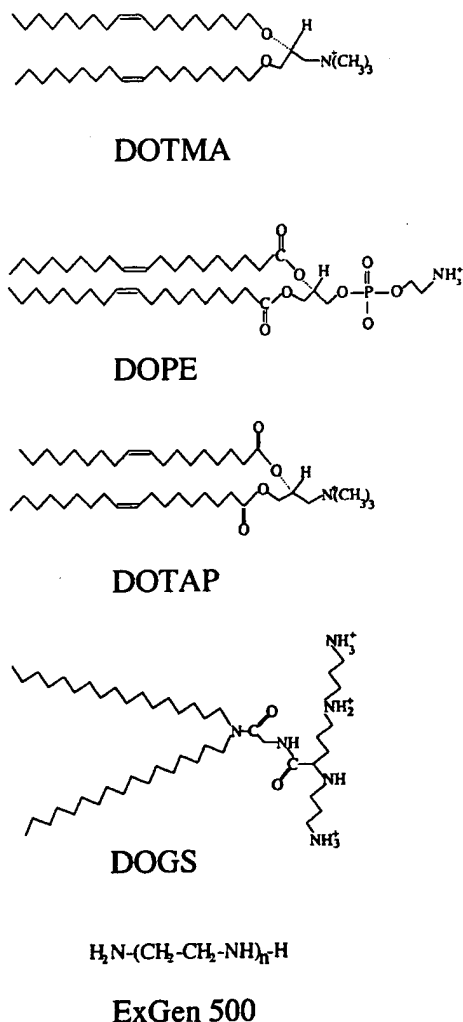


Figure 1 Chemical structures of the synthetic compounds used for *in vitro* and *in vivo* experiments. DOTMA, 1,2-dioleoyloxypropyl-3-trimethyl ammonium bromide; DOPE, dioleoylphosphatidylethanolamine. The 1:1 (w/w) association of DOTMA and DOPE is Lipofectin. DOTAP, 1,2-dioleoyloxypropyl-3-(trimethylammonium)propane; DOGS, dioctadecylamidoglycylspermine (Transfectam); ExGen 500, a 22 kDa linear form of polyethylenimine, PEI.

(CFPEo⁻, CFNPEo⁻ and 6CFSMEo⁻) and normal trachea cells (9HTEo⁻).^{16,17} Transfections with Lipofectin, DOTAP and DOGS were performed using positively charged complexes since it has been shown that *in vitro* transfection with cationic lipids is best when the nucleolipid particles bear strong net positive charge.^{12,18-19}

Figure 2 shows that DOGS is more efficient than Lipofectin and DOTAP in nasal polyp and tracheal cells, reaching values ranging from 2×10^7 to 10^8 RLU/10 s/mg of protein. DOGS's efficiency may be due to its least basic secondary amine ($pK_a = 5.4$) which is able to buffer acidic endosomes and protect DNA from degradation.¹⁸ On the other hand, for reasons that have still to be understood, DOGS is less efficient on submucosal gland cells, reaching values of 2×10^7 RLU/10 s/mg of protein. This result should be taken into consideration when *in vivo* experiments are planned, since cell transfection variability might compromise their outcome. In fact, submucosal

gland cells are active in producing sticky mucus in CF patients and should be the target cells to be corrected by gene transfer. In contrast, ExGen 500 showed no cell transfection variability, being significantly more efficient than Lipofectin and DOTAP ($P < 0.05$) and DOGS ($P < 0.025$) even in submucosal cells.

ExGen 500 complexes were characterized on the basis of their PEI nitrogen per DNA phosphate ratio (N/P) and expressed as ExGen 500 equivalents. Several ratios were tested (9:1, 5:1, 1:1). Assuming linear PEI-DNA complexes to have protonation profiles similar to that of branched PEI,¹⁴ neutral complexes should be obtained for a N:P ratio of 5. Similar transfection values were obtained both with neutral (five equivalents) and positively charged complexes (nine equivalents), although signs of acute toxicity (cell detachment and death) were observed only when positively charged complexes were used (data not shown). In contrast, low transfection efficiency was obtained at negative ratios (10^5 RLU/10 s/mg of protein). Figure 2 shows that ExGen 500, at a neutral charge ratio, was more efficient than cationic lipids in transfecting epithelial cells *in vitro*, with values close to 10^9 RLU/10 s/mg of protein.

ExGen 500's high transfection efficiency might be explained by the 'proton sponge' effect which leads to osmotic swelling of endosomes containing the complexes.²⁰ However, the 'proton sponge' behaviour is not the only characteristic which makes PEI attractive for gene therapy purposes. Another major property of PEI is the possibility of reaching high efficiency of transfection by using complexes with a charge ratio close to neutrality. This might be advantageous for *in vivo* applications. Indeed, cationic lipid-DNA complexes bearing a strong positive charge do not mediate efficiently the gene transfer *in vivo*, since interaction with circulating serum proteins or with the anionic tissue matrix hinders their bio-availability.²¹ Furthermore, positively charged complexes also activate complement and complement-dependent phagocytosis by macrophages in the reticuloendothelial system.²²

Neutral ExGen 500-DNA complexes were compared to DOGS-DNA complexes in newborn rabbits (5 days old, 100 g weight). The plasmid DNA (pCMV-luciferase or pCMV-nlslacZ) was mixed with DOGS or ExGen 500 in 300 μ l 5% sucrose solution and injected directly into the newborn rabbits' trachea. Forty-eight hours after injection, the animals were killed and trachea and lung lobes were analysed for luciferase activity or β -galactosidase expression by X-gal staining.

When 100, 300 and 700 μ g DNA were complexed to DOGS to form positively charged complexes (6:1 charge ratio), none of the 30 animals was positive for luciferase activity or β -galactosidase expression. On the other hand, when DOGS, in association with DOPE, was used at neutral conditions (1:1 DOGS-DOPE:DNA charge ratio) and complexed to 1 mg DNA, some animals (four out of 60) were positive for β -galactosidase staining. Transfection efficiency was low ($<0.01\%$) and restricted to the injection site (data not shown), as previously described in mice.¹³

In sharp contrast to DOGS, ExGen 500 was able to deliver DNA efficiently and reproducibly. Twenty-four newborn rabbits were analysed for β -galactosidase expression following instillation of ExGen 500-DNA complexes at neutral, negative and positive surface

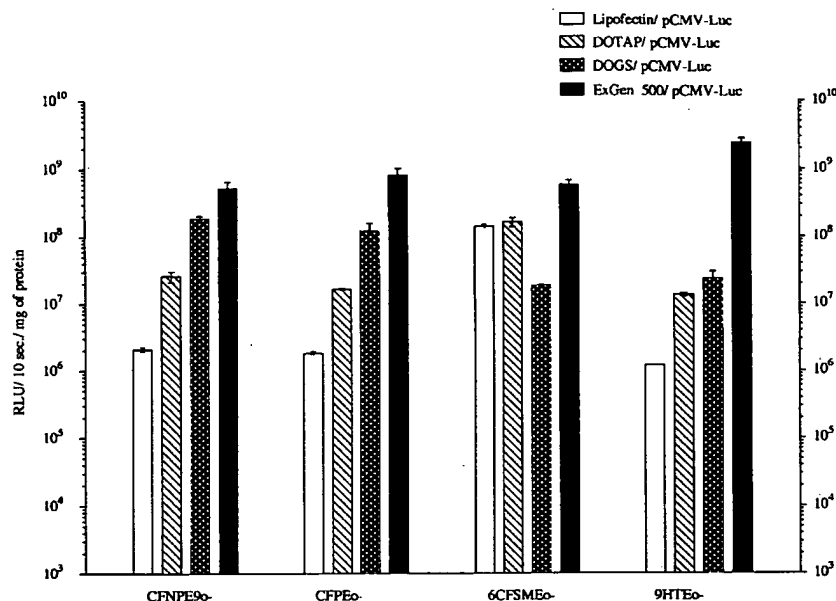


Figure 2 In vitro transfection of lung epithelial cells with synthetic compounds. Transfection efficiency was tested on transformed respiratory cell lines (9HTEo⁻, CFPEo⁻, CFNPE9o⁻ and 6CFSMEo⁻), kindly provided by Dr DC Gruenert (San Francisco, University of California, CA, USA). CF cell lines CFNPE9o⁻, CFPEo⁻ and 6CFSMEo⁻ were obtained from nasal epithelium, trachea and submucosal glands, respectively. 9HTEo⁻ derived from normal adult trachea epithelium. Cells were maintained in 1:1 DMEM/F12 (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% FCS (Boehringer Mannheim, Mannheim, Germany), 2 mM L-glutamine (GIBCO BRL), 100 units/ml penicillin (GIBCO BRL) and 100 µg/ml streptomycin (GIBCO BRL) at 37°C in a 5% CO₂/95% air humidified atmosphere. The pCMV-luciferase (pCMV-Luc) used for transfections was constructed by substituting the E. coli lacZ gene of pCMV-βgal²⁵ with the P. pyralis luciferase (Luc) gene. The 1704 bp Luc gene was isolated from pRSV-Luc²⁶ by restriction with SmaI-HindIII, the HindIII site was filled in by Klenow reaction. The pCMV vector was obtained by excision of the lacZ gene with EcoRV and NotI from pCMV-βgal, the NotI site was filled in by Klenow reaction. The Luc gene was then cloned into the pCMV vector by T4 ligase reaction. Twenty-four hours before transfection cells were counted and seeded at a density of 8 × 10⁴ cells per well in a 12-well plate. Each well received a total of 2 µg pCMV-Luc. Transfections with Lipofectin (GIBCO BRL) and DOTAP (Boehringer Mannheim) were set up following optimal conditions suggested by the manufacturers' protocols. Transfection conditions with DOGS (Transfectam; Promega, Madison, WI, USA) were set up as previously described.²⁷ The amount of cationic lipid used for the transfection is defined here as charge equivalent. One equivalent is the amount of lipid able to neutralize the negative phosphate charges of 1 µg DNA (3 nmol): 7.5 µg Lipofectin (1.7 equivalents), 6 µg DOTAP (2.5 equivalents) and 7.5 µg DOGS (6 equivalents) were used for each microgram of DNA. Transfections were set up in serum-free medium: after 3–4 h, the medium was supplemented with 10% FCS. ExGen 500 (Euromedex, Souffelweiersheim, France) was used as a 10 mM stock solution: 1.5 µl (= 5 N/P equivalents) were used for each microgram of DNA. The pCMV-Luc (2 µg) and ExGen 500 were each diluted into 50 µl of 150 mM NaCl solution and vortexed. After 10 min, the two solutions were mixed and vortexed. After 15 min more, the transfection mixture was added to the cells. Transfection was set up in 10% FCS medium. Luciferase gene expression was monitored 24 h later by using the Luciferase Assay System (Promega) and photon counting. Each transfection experiment was performed in triplicate and expressed as the mean of relative light units per 10⁵ cells per mg of cell protein (measured by Bradford method) ± s.d. A statistical analysis of the data was performed using an analysis of variance (ANOVA) test (see text for details).

charge. Forty-eight hours after gene transfer, rabbit lungs were removed *en bloc* and stained for lacZ activity. In all 24 animals, X-gal staining produced a predominantly bilateral perihilar pattern of reporter gene expression (Figure 3a). No X-gal staining was observed in control animals.

In order to evaluate the histologic distribution of reporter gene expression, 10–15 frozen sections were cut from each lung lobe of each injected animal. When neutral complexes (N/P = 5) were injected, β-galactosidase expressing cells were detected in the trachea (Figure 3b) as well as around the lumen of large and small bronchi (Figure 3d and e). Blue cells were found in upper and lower lung lobes and we estimated that 1–2% of the cells in each section were positive to X-gal staining. By using this condition, in fact, increased particles' half-life and a consequently higher probability of reaching distant cells was expected.

When negatively charged complexes (N/P = 1) were injected, transfection efficiency was restricted to tracheal cells and no β-galactosidase expressing cells were found in lung sections. This might be due to a reduced DNA

protection from degradation before reaching the distal lung cells.

On the other hand, high levels of toxicity became evident when positively charged complexes (N/P = 9) were used. Lung sections from rabbits treated with high amounts of ExGen 500 showed the presence of mononuclear and red blood cell infiltration (Figure 3f), which was not visible at lower N/P ratios.

In order to evaluate the dose dependency of *in vivo* transfection, 100, 300 and 700 µg DNA were mixed with ExGen 500 at a neutral (N/P = 5) ratio in 300 µl 5% sucrose solution and injected directly into the newborn rabbits' tracheae. Forty-eight hours later, rabbits were killed; trachea and lung lobes were excised and transfection efficiency was quantified by a luciferase assay. No activity was detected when 100 µg DNA were used. Figure 4 shows that no significant difference ($P < 0.2$) in luciferase activity was detected when 300 or 700 µg DNA were injected (average 10³ RLU/10 s/mg of protein). Similar experiments performed by using the pCMV-nlslacZ construct confirmed *de visu* that no difference in the number of transfected cells could be observed follow-

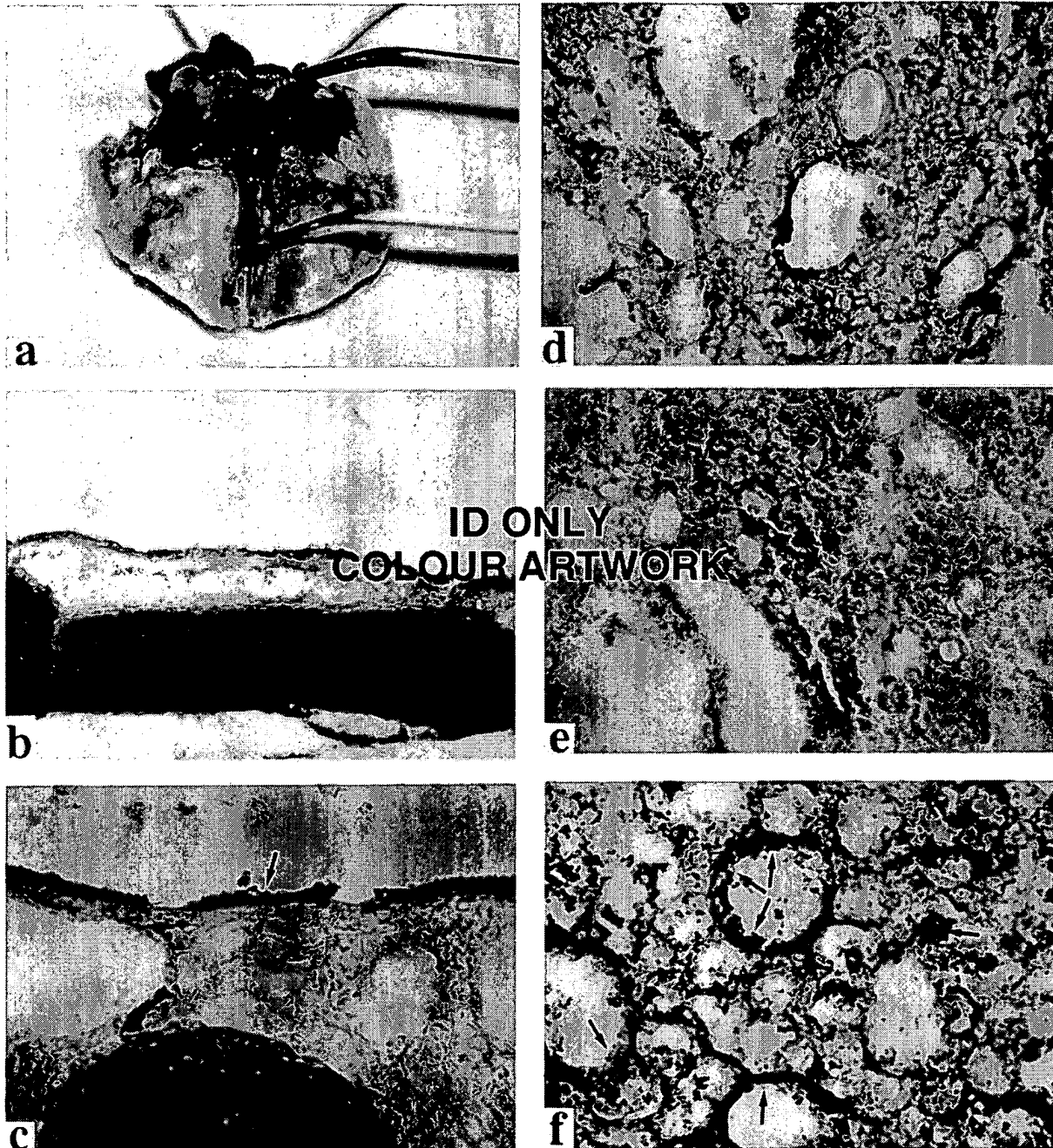


Figure 3 β -Galactosidase gene transfer in vivo. The pCMV-nlslacZ plasmid (300 or 700 μ g) and the desired amount of ExGen 500 were each diluted into 150 μ l 5% sucrose solution, vortexed and mixed after 10 min. Twenty minutes later, the complexes were injected. A maximum volume of 300 μ l was injected into newborn rabbits to avoid a volume-dependent respiratory insufficiency. Neutral complexes were formed by mixing 300 and 700 μ g DNA to 4.5 and 10.5 μ l of 1 M ExGen 500, respectively. Plasmid DNA was prepared by using a pZ523 Column kit (5 Prime-3 Prime, Boulder, CO, USA). Twenty-four newborn rabbits (4 to 5 days old and 100 g weight) were used. As control, six animals were injected with 700 μ g naked DNA resuspended in 300 μ l 5% sucrose solution. The trachea was exposed through a 0.5 cm longitudinal skin incision in the neck and the transfection mixture was injected via a 28 G needle into the distal trachea. The site was then closed with two sutures. After 48 h, rabbits were killed by injection of a lethal dose of penthotal sodium intraperitoneally. Heart, trachea and lungs were excised en bloc and used for in toto X-gal staining or for histochemistry on sections. After washing twice with ice-cold PBS, organs were infused with an ice-cold fixing solution containing 2% paraformaldehyde/0.2% glutaraldehyde in Na-phosphate buffer pH 7.3. After 4 h fixation at 4°C, lungs and trachea were washed with ice-cold PBS and perfused with the X-gal staining solution (1.3 mM $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$ with 1 mg/ml X-gal (ICN Pharmaceuticals, Costa Mesa, CA, USA) in Na-phosphate buffer pH 7.3) overnight at 37°C. For histochemistry on sections, after in toto X-gal staining, trachea and lung lobes were separated, embedded in OCT compound (Miles, Elkhart, IN, USA) and immediately frozen in liquid nitrogen. The frozen pieces were sectioned at 5–10 μ m, mounted on to glass slides and stained with hematoxylin and eosin. Blue cells were detected by direct light microscopy. (a) Overall view of β -galactosidase expression in toto; (b) section showing transfected cells in the trachea of animals receiving ExGen 500-DNA neutral complexes ($\times 100$); (c) section showing transfection of trachea cells in animals injected with naked DNA ($\times 250$); (d and e) sections showing transfected epithelial cells in the lung, around the lumen of large and small bronchi respectively when ExGen 500-DNA neutral complexes were injected ($\times 160$); (f) toxic effect and cell infiltration (see arrows) due to transfection with positively charged complexes (obtained by mixing 300 or 700 μ g DNA with 8.1 and 18.9 μ l of 1 M ExGen 500, respectively) ($\times 160$).

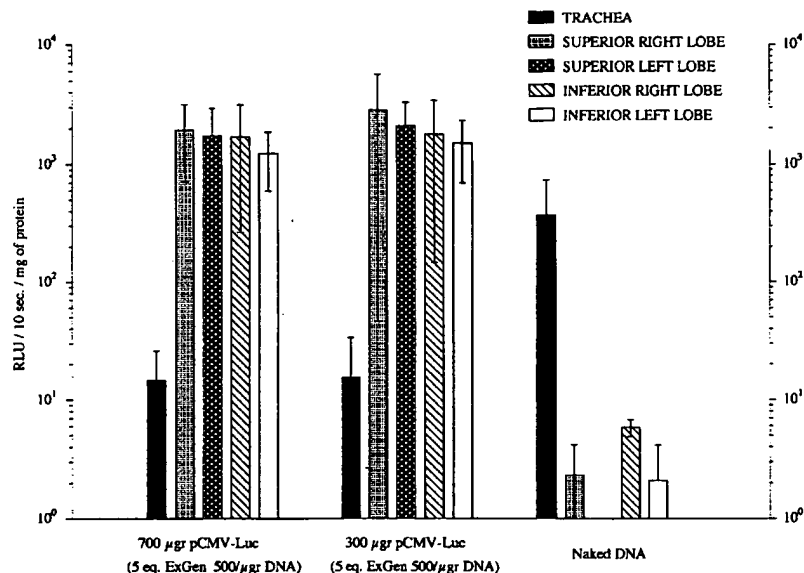


Figure 4 Quantification of the *in vivo* transfection efficiency mediated by ExGen 500 in newborn rabbits. Three groups of 15 animals received 100, 300 and 700 µg of pCMV-Luc complexed with ExGen 500 at five equivalents N/P in 300 µl 5% sucrose solution. As control, six animals received 700 µg of naked plasmid DNA resuspended in 300 µl 5% sucrose solution. Complexes formation and instillation were performed as described in Figure 3. After 48 h, rabbits were killed by injection of a lethal dose of penthotal sodium intraperitoneally. The trachea and each lobe of the lung were separately homogenized in 500 µl of ice-cold lysis buffer (Promega) and then centrifuged. Next, 20 µl of the supernatant were mixed with 100 µl of luciferase reagent (Promega) and the light emitted over 10 s was measured in a single-well luminometer. Results are expressed per group (15 animals) and represented as relative light units/mg of protein per 10 s \pm s.d. After applying an ANOVA test to establish differences between means, a Student's *t* test was used. The graph regarding the transfection values obtained when 100 µg DNA were injected is not shown, since no luciferase activity was detected.

ing the injection of 300 µg or 700 µg DNA (data not shown). These results might be due to the clumping of complexes which was observed at concentration >1 µg/µl. This phenomenon in turn would result in a decreased diffusibility of complexes through the extracellular space as well as in a reduced probability of being endocytosed.

To ascertain further whether *in vivo* transfection of lung epithelial cells was mediated by ExGen 500, control animals were injected with naked DNA. Indeed, it was shown recently that naked DNA was as effective as lipo-

some-DNA complexes in transfecting mouse airway epithelial tissue.²³ From the data shown in Figure 4, it was evident that ExGen 500 was essential to mediate gene transfer into rabbit lungs: naked DNA was unable to deliver DNA to airways cells (<10 RLU/10 s/mg of protein). However naked DNA was able to transfect tracheal cells to some extent (average 4×10^2 RLU/10 s/mg of protein) and X-gal staining was observed around the site of injection (Figure 3c).

Based on these observations, to demonstrate that adult animals could be transfected as well as newborns, we injected 300 or 700 µg DNA at 1 µg/µl concentration into 30-day-old rabbits (1.5 kg weight). As shown in Figure 5, 700 µg of pCMV-luciferase led to similar transfection values (10^3 RLU/10 s/mg of protein) as those obtained in newborn rabbits ($P < 0.2$), with no apparent toxicity. The absence of luciferase activity in the heart, liver and gonads confirmed that intratracheal injection was an appropriate technique to restrict distribution of DNA to the lung.

A drastic decrease (97%) of luciferase activity was observed 1 week after injection (Table 1). Our data correlate well with the data of Sawa *et al*,²⁴ who also observed a decrease in the level of luciferase expression 7 days after instillation of the plasmid in rats; this indicates the need for further experiments to set up conditions allowing prolonged expression.

In conclusion, our data indicate that ExGen 500 might be a promising nonviral vector worthy of testing in non-human primates and in a phase I clinical trial in order

Table 1 Detection of luciferase activity 48 h and 7 days after injection

	48 h after injection LU/10 s/total lysate	1 Week after injection LU/10 s/total lysate
Trachea	76 \pm 41	75 \pm 37
Right superior lobe	41975 \pm 22828	937 \pm 398
Left superior lobe	43223 \pm 20828	335 \pm 127
Right inferior lobe	37215 \pm 25587	272 \pm 105
Left inferior lobe	40068 \pm 18450	413 \pm 232

For materials and methods, see legend for Figure 3. Results are expressed as light units (LU) per 10 s on total lung lobe (or trachea) lysate \pm s.d.

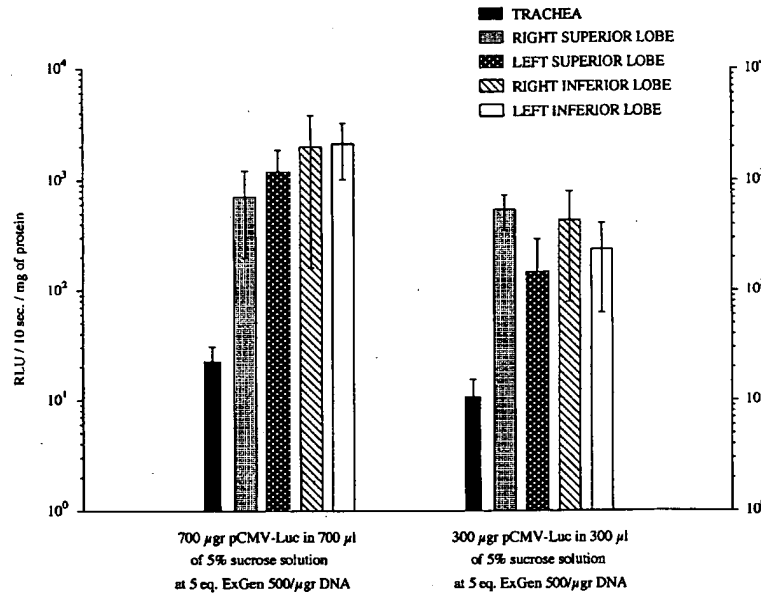


Figure 5 Quantification of the *in vivo* transfection efficiency mediated by ExGen 500 in adult rabbits. Plasmid DNA preparation and complexes formation were carried out as described in Figure 3. Neutral charged complexes were obtained by mixing 300 or 700 µg of pCMV-Luc with ExGen 500 in a 5% sucrose solution at 1 µg/µl concentration. A maximum volume of 700 µl was injected to avoid a volume-dependent respiratory insufficiency. Twelve adult rabbits (30 days old and 1.5 kg weight) were used. For the instillation of the complexes, animals were anesthetized by injection of 0.5 ml of 1:1 (v/v) Ketalar/Rompun (Parke-Davis, Milan, Italy; Bayer, Leverkusen, Germany). Injection of complexes was performed as described in Figure 3. After 48 h, rabbits were killed by an intravenous injection of a lethal dose of penthotal sodium. Trachea and lung lobes were homogenized as described in Figure 4, but 1 ml ice-cold lysis buffer was used. Luciferase analysis, light unit measurements and statistical analysis were carried out as described in Figure 4.

to demonstrate its efficacy for the gene therapy of lung-related disorders.

Acknowledgements

This work was supported in part by the Association Française pour la Lutte contre la Mucoviscidose (AFLM), Paris, France and by a special CF grant of the Italian Minister of Health. We wish to thank Dr DC Gruenert, who kindly provided the epithelial cell lines used in this study, and Drs MA Zanta and O Boussif for helpful discussions.

References

- Rommens JM *et al.* Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; 245: 1059-1065.
- Riordan JR *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066-1073.
- Zabner J *et al.* Repeat administration of an adenovirus vector encoding cystic fibrosis transmembrane conductance regulator to the nasal epithelium of patients with cystic fibrosis. *J Clin Invest* 1996; 97: 1504-1511.
- Fisher KJ *et al.* Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. *Virology* 1996; 217: 11-22.
- Caplen NJ *et al.* Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nature Med* 1995; 1: 39-46.
- Sorscher EJ *et al.* Clinical protocol. Gene therapy for cystic fibrosis using cationic liposome-mediated gene transfer: a phase I trial of safety and efficacy in the nasal airway. *Hum Gen Ther* 1994; 5: 1259-1277.
- Yoshimura K *et al.* Expression of the human cystic fibrosis transmembrane conductance regulator gene in the mouse lung after *in vivo* intratracheal plasmid-mediated gene transfer. *Nucleic Acids Res* 1992; 20: 3233-3240.
- Logan JJ *et al.* Cationic lipids for reporter gene and CFTR transfer to rat pulmonary epithelium. *Gene Therapy* 1995; 2: 38-49.
- Zhu N, Liggitt D, Lui Y, Debs R. Systemic gene expression after intravenous DNA delivery into adult mice. *Science* 1993; 261: 209-211.
- Hyde SC *et al.* Correction of ion transport defect in cystic fibrosis transgenic mice by gene therapy. *Nature* 1993; 362: 250-255.
- McLachlan G *et al.* Evaluation *in vitro* and *in vivo* of cationic liposome-expression construct complexes for cystic fibrosis gene therapy. *Gene Therapy* 1995; 2: 614-622.
- Behr JP, Demeneix B, Loeffler JP, Perez MJ. Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc Natl Acad Sci USA* 1989; 86: 6982-6986.
- Fortunati E *et al.* *In vitro* and *in vivo* gene transfer to pulmonary cells mediated by cationic liposomes. *Biochim Biophys Acta* 1996; 1306: 55-62.
- Boussif O *et al.* A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine. *Proc Natl Acad Sci USA* 1995; 92: 7297-7301.
- Abdallah B *et al.* A powerful nonviral vector for *in vivo* gene transfer into the adult mammalian brain: polyethylenimine. *Hum Gen Ther* 1996; 7: 1947-1954.
- Gruenert DC *et al.* Characterization of human tracheal epithelial cells transformed by an origin-defective simian virus 40. *Proc Natl Acad Sci USA* 1988; 85: 5951-5955.
- Cozens AL *et al.* Characterization of immortal cystic fibrosis tracheobronchial gland epithelial cells. *Proc Natl Acad Sci USA* 1992; 89: 5171-5175.
- Behr JP. Gene transfer with synthetic cationic amphiphiles: prospects for gene therapy. *Bioconj Chem* 1994; 5: 382-389.
- Remy JS *et al.* Targeted gene transfer into hepatoma cells with lipopolyamine-condensed DNA particles presenting galactose ligands: a stage toward artificial viruses. *Proc Natl Acad Sci USA* 1995; 92: 1744-1748.



- 20 Behr JP. L'éponge à protons: un moyen d'entrer dans une cellule auquel les virus n'ont pas pensé. *Médecine/Sciences* 1996; 12: 56-59.
- 21 Schwartz B *et al*. Lipospermine-based gene transfer into the newborn mouse brain is optimized by a low lipospermine/DNA charge ratio. *Hum Gen Ther* 1995; 6: 1515-1524.
- 22 Plank C, Mechtler K, Szoka FC, Wagner E. Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. *Hum Gen Ther* 1996; 7: 1437-1446.
- 23 Meyer KB *et al*. Intratracheal gene delivery to the mouse airway: characterization of plasmid DNA expression and pharmacokinetics. *Gene Therapy* 1995; 2: 450-460.
- 24 Sawa T *et al*. Intraluminal water increases expression of plasmid DNA in rat lung. *Hum Gene Ther* 1996; 7: 933-941.
- 25 MacGregor GR, Caskey CT. Construction of plasmids that express *E. coli* β -galactosidase in mammalian cells. *Nucleic Acids Res* 1989; 17: 2365.
- 26 de Wet JR *et al*. Firefly luciferase gene: structure and expression in mammalian cells. *Mol Cell Biol* 1987; 7: 725-737.
- 27 Barthel F, Remy JS, Loeffler JP, Behr JP. Gene transfer optimization with lipospermine-coated DNA. *DNA Cell Biol* 1993; 12: 553-560.